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TITLE: Optimizing Hemodynamic Support of Acute Spinal Cord Injury Based on Injury Mechanism

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

In Year 2 we finalized the head-to-head comparison of NE, and PE infusion after SCI on spinal cord oxygenation (luminescence-based optical sensor), perfusion (laser-Doppler flowmetry), intraparenchymal pressure (fiber optic probe) and downstream metabolic responses (microdialysis). Correlation analysis revealed that changes in MAP during NE and PE in the compressed state of the spinal cord were not associated with positive changes in neither SCBF nor $PaPO_2$. Notably during the decompressed state, there was no relation between changes in MAP and SCBF with PE, while an almost linear correlation existed with the use of NE. Notably, glutamate and L/P ratio levels were significantly lower with the use of NE during decompression compared to no infusion, 1 hour after infusion had stopped indicative of reduced glutamate toxicity and ischemia. This is most likely due to the improved perfusion after decopresion as observed with NE. These results suggest that NE may be preferable to PE if vasopressor support is required post SCI to maintain elevated MAPs in accordance with published guidelines.

15. SUBJECT TERMS

hemodynamic support, SCI, vasopressors, blood flow, oxygenation, pressure

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1 INTRODUCTION

There are currently very few treatments to improve the neurologic outcome of individuals who sustain an acute spinal cord injury (SCI). Treatment options include urgent surgical decompression to relieve pressure on the spinal cord and aggressive augmentation of systemic blood pressure to minimize ischemia. However, improved outcomes from these approaches have not been convincingly demonstrated in randomized or controlled clinical trials, and hence they are not considered 'standards of care'. We postulate that the difficulty in unequivocally demonstrating the benefits of aggressive hemodynamic support may be due to this approach eliciting not only beneficial but also adverse effects on the injured spinal cord, depending upon the presence or absence of spinal cord compression. Our overall objective is therefore to determine how hemodynamic support of mean arterial pressure (MAP) in the presence or absence of spinal cord compression affects the vascular, metabolic, biochemical, and behavioral outcomes of traumatic SCI. We hypothesize that well-intended increases in MAP after decompression contribute to detrimental edema/swelling, hemorrhage, and increased intraparenchymal pressure, in addition to exacerbating ischemia-reperfusion injury mechanisms. To test this hypothesis, we will utilize a novel pig model of SCI, for which we have developed innovative techniques for measuring intraparenchymal spinal cord blood flow (SCBF), hydrostatic pressure, and metabolic responses over time. Achieving a better understanding of how the spinal cord responds to alterations in MAP before and after decompression will provide insights that could be deployed rapidly into clinical practice to optimize the hemodynamic management of acute SCI. Such insights have added significance for soldiers injured in combat where sophisticated therapies are likely not available and the early treatment of their SCI may be limited to basic hemodynamic resuscitation and the management of their MAP.

2 KEYWORDS

- Hemodynamic Support
- Spinal Cord Injury Based
- Cord Compression
- Cord Decompression
- Porcine model of SCI
- Spinal Cord Blood Flow
- Microdialysis
- Intraparenchymal Pressure
- Vasopressors

3 ACCOMPLISHMENTS

3.1 Protocol and Activity Status

- **Human Use Regulatory Protocols**
  No human subjects' research will be performed to complete the Statement of Work

- **Use of Human Cadavers for RDT&E, Education or Training**
  No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work

- **Animal Use Regulatory Protocols**

  Total Protocols: 1 animal use research protocol will be required to complete the Statement of Work

- **Protocol:** 1 of 1
- **Protocol [ACURO Assigned Number]:** SC130007
- **Title**: SCI in pigs [IACUC protocol number A13-0013]
- **Target required for statistical significance**: n=6/group
- **Target approved for statistical significance**: n=6/group
- **Submitted to and Approved by**: Bryan K. Ketzenberger, DVM, DACLAM
- **Status:** Approved, 26-March-2015

3.2 Approved Statement of Work

The approved statement of work is described below. A Gantt chart is provided in Table 1 for reference (see page 5).

Aim 1. Determine the acute vascular, metabolic, and pressure responses to SCI and long-term functional outcome. "SCI with no hemodynamic support"

Task 1: Submit documents for ACURO approval [Month(s) 1-3]
Task 2: Characterize the intraparenchymal metabolic, blood flow, and pressure changes that occur during the first 24 hours post-injury with no hemodynamic resuscitation before or after decompression [Month(s) 4-18]
Task 3: Determine the long-term functional and histologic outcome of SCI with no hemodynamic resuscitation before or decompression [Month(s) 16-36]
Task 4: Evaluate the metabolomic changes that occur within the spinal cord and surrounding CSF and compare these with metabolomic changes within human CSF [Month(s) 7-15 and 22-33]

Milestone(s) Achieved:
(a) Determine baseline intraparenchymal responses to SCI without treatment [Month(s) 18]
(b) Determine how invasive monitoring alone influences functional recovery [Month(s) 36]

Aim 2. Determine the effects of aggressive hemodynamic support during sustained spinal cord compression on the acute physiologic responses to spinal cord injury and long-term functional outcome. "SCI with SCI with hemodynamic support during compression"

Task 1: Characterize the intraparenchymal metabolic, blood flow, and pressure changes that occur during the first 24 hours post-injury with aggressive hemodynamic support provided during sustained spinal cord compression [Month(s) 4-18]

Task 2: Determine the long-term functional and histologic outcome of SCI when aggressive hemodynamic support is provided during sustained compression [Month(s) 16-36]

Task 3: Evaluate the metabolomic changes that occur within the spinal cord and surrounding CSF and compare these with metabolomic changes within human CSF [Month(s) 7-15 and 22-33]

Milestone(s) Achieved:
(a) Determine if hemodynamic support during spinal cord compression can restore perfusion and alleviate intraparenchymal ischemia [Month(s) 18]
(b) Determine if hemodynamic support during spinal cord compression improves functional recovery [Month(s) 36]

Aim 3. Determine the effects of aggressive hemodynamic support after spinal cord decompression on the acute physiologic responses to spinal cord injury and long-term functional outcome.

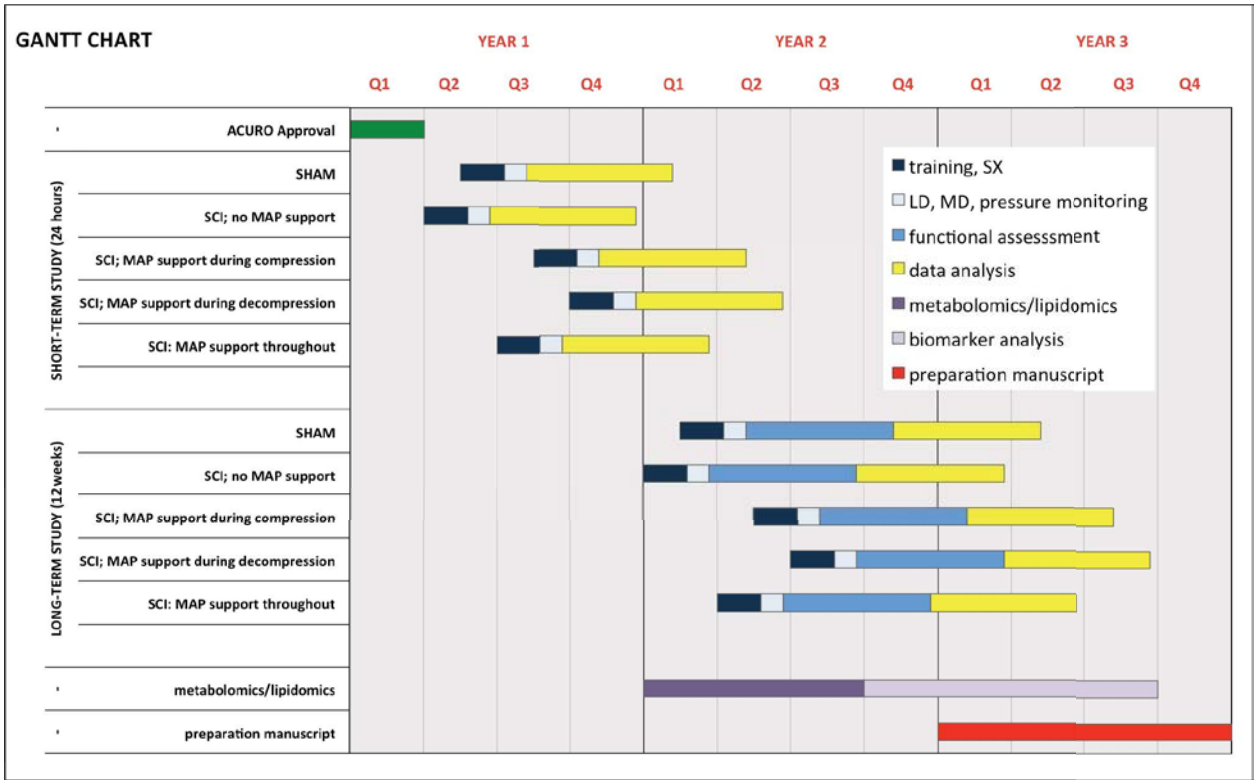
Task 1: Characterize the intraparenchymal metabolic, blood flow, and pressure changes that occur during the first 24 hours post-injury with aggressive hemodynamic support provided after spinal cord decompression [Month(s) 16-36]

Task 2: Determine the long-term functional and histologic outcome of SCI when aggressive hemodynamic support is provided after cord decompression [Month(s) 7-15 and 22-33]

Task 3: Evaluate the metabolomic changes that occur within the spinal cord and surrounding CSF and compare these with metabolomic changes within human CSF [Month(s) 16-36]

Milestone(s) Achieved:
(a) Determine if hemodynamic support after spinal cord decompression induces an ischemia-reperfusion injury [Month(s) 18]
(b) Determine if hemodynamic support after spinal cord compression worsens functional recovery [Month(s) 36]

Table 1. Approved statement of work (Gantt Chart)



3.3 Current Progress on Statement of Work

A Gantt chart of the current work is provided in Table 2 for reference (page 7). The months in the approved statement of work (Table 1) do not necessarily match with the Gantt chart (Table 2), since the latter reflects actual work completed in each year.

Aim 1. Determine the acute vascular, metabolic, and pressure responses to SCI and long-term functional outcome. "SCI with no hemodynamic support"

- **Task 1:** Submit documents for ACURO approval

  Completed. *ACURO approval was granted 26-MARCH-2015.*

- **Task 2:** Characterize the intraparenchymal metabolic, blood flow, and pressure changes that occur during the first 24 hours post-injury with no hemodynamic resuscitation before or after decompression

  ***Completed*.**

- **Task 3:** Determine the long-term functional and histologic outcome of SCI with no hemodynamic resuscitation before or decompression

  In progress.

- **Task 4:** Evaluate the metabolomic changes that occur within the spinal cord and surrounding CSF and compare these with metabolomic changes within human CSF

  In progress.

Aim 2. Determine the effects of aggressive hemodynamic support during sustained spinal cord compression on the acute physiologic responses to SCI and long-term functional outcome. "SCI with SCI with hemodynamic support during compression"

- **Task 1:** Characterize the intraparenchymal metabolic, blood flow, and pressure changes that occur during the first 24 hours post-injury with aggressive hemodynamic support provided during sustained spinal cord compression

  In progress.

- **Task 2:** Determine the long-term functional and histologic outcome of SCI when aggressive hemodynamic support is provided during sustained compression

  In progress.

- **Task 3:** Evaluate the metabolomic changes that occur within the spinal cord and surrounding CSF and compare these with metabolomic changes within human CSF

  In progress.

Aim 3. Determine the effects of aggressive hemodynamic support after spinal cord decompression on the acute physiologic responses to spinal cord injury and long-term functional outcome.

- **Task 1:** Characterize the intraparenchymal metabolic, blood flow, and pressure changes that occur during the first 24 hours post-injury with aggressive hemodynamic support provided after spinal cord decompression

  In progress.

- **Task 2:** Determine the long-term functional and histologic outcome of SCI when aggressive hemodynamic support is provided after cord decompression

  In progress.

- **Task 3:** Evaluate the metabolomic changes that occur within the spinal cord and surrounding CSF and compare these with metabolomic changes within human CSF

  In progress.

Table 1: Gantt chart of current work. The Gantt chart reflects actual work completed. Therefore, months in the approved statement of work do not necessarily match with the Gantt chart, since the Gantt chart reflects actual work completed in each year.

Specific Aim 1: *SCI with no hemodynamic support* 1a. 24hr acute physiologic experiment (n=6) 1b. 12w functional experiment (n=6)	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
• ACURO Approval												
• 1a. Animal surgery / 24hr physiologic assessments												
• 1a. Metabolomic / Histologic assessments												
• 1a. Data analysis / dissemination												
• 1b. Animal training / Surgery												
• 1b. Behavioral / functional assessments												
• 1b. Metabolomic / Histologic assessments												
• 1b. Data analysis / dissemination												

Specific Aim 2: *SCI with hemodynamic support during compression* 2a. 24hr acute physiologic experiment (n=6) 2b. 12w functional experiment (n=6)	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
• 2a. Animal surgery / 24hr physiologic assessments												
• 2a. Metabolomic / Histologic assessments												
• 2a. Data analysis / dissemination												
• 2b. Animal training / Surgery												
• 2b. Behavioral / functional assessments												
• 2b. Metabolomic / Histologic assessments												
• 2b. Data analysis / dissemination												

Specific Aim 3: *SCI with hemodynamic support after decompression* 3a. 7d acute physiologic experiment (n=6) 3b. 12w functional experiment (n=6)	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
• 3a. Animal surgery / 24hr physiologic assessments												
• 3a. Metabolomic / Histologic assessments												
• 3a. Data analysis / dissemination												
• 3b. Animal training / Surgery												
• 3b. Behavioral / functional assessments												
• 3b. Metabolomic / Histologic assessments												
• 3b. Data analysis / dissemination												

4 OVERALL PROJECT SUMMARY

This grant is focused on the augmentation of MAP with vasopressors and determining how such MAP augmentation would best be implemented (during compression, after decompression, or both). One of the issues that we felt needed to be addressed at the outset was to determine which vasopressor to use in order to actually accomplish this MAP augmentation. In Year 2 of this grant we finalized a head-to-head comparison of two commonly used vasopressors, norepinephrine (NE) and PE (PE) after SCI to determine how these commonly used vasopressors affects spinal cord oxygenation (luminescence-based optical sensor), perfusion (laser-Doppler flowmetry), intraparenchymal pressure (fiber optic probe) and downstream metabolic responses (microdialysis) (See Appendix 1, page 18 for manuscript draft). For this study we used female Yorkshire pigs subjected to a combination of contusion trauma and compression at the T10 level.

Using PE or NE, MAP was augmented by 20 mmHg above pre-SCI levels. reaching target levels of around 75-85 mmHg. In summary, MAP augmentation of ~20 mm Hg was achieved comparably between PE and NE. Correlation analysis revealed that changes in MAP during NE and PE in the compressed state of the spinal cord were not associated with positive changes in neither SCBF nor PaPO2. Notably during the decompressed state, there was no relation between changes in MAP and SCBF with PE, while an almost linear correlation existed with the use of NE. Additionally, an augmentation of SCBF correlated directly to positive changes in PaPO2 using NE, which was contrary to PE. Notably, glutamate and L/P ratio levels were significantly lower with the use of NE during decompression compared to no infusion, 1 hour after infusion had stopped indicative of reduced glutamate toxicity and ischemia. This is most likely due to the improved perfusion after decompression as observed with NE. These results suggest that NE may be preferable to PE if vasopressor support is required post SCI to maintain elevated MAPs in accordance with published guidelines. See Appendix 1, page 18 for manuscript draft.

4.1 Results

We recently finalized the histological analysis of the spinal cords of these animals (Eriochrome Cyanine R Staining). After collection of the spinal cords, segments were cryoprotected using a graded concentrations of sucrose (6, 12, 24% sucrose in 0.1 M Phosphate Buffer each for 48 hours). The tissue was then embedded in Tissue-TekTM CRYO-OCT compound (Sakura Finetek, Torrance, CA) and frozen before being cut into serial 20 µm thick transverse sections using a cryostat. Every other section was mounted on a series of 10x silane coated slides, such that the adjacent sections mounted on each slide represented regions spaced 400 µm apart. For differentiating

grey and white matter, Eriochrome Cyanine R staining was performed on spinal cord sections, which specifically stains myelin sheaths blue. Briefly, sections were dehydrated in an ethanol series, cleared in xylene, rehydrated in a reverse ethanol series followed by distilled water (dH_2O), then left in a solution containing 0.16% Eriochrome Cyanine R, 0.5% sulphuric acid and 0.4% iron chloride (in dH_2O) to stain myelinated fibres. Following staining, sections were differentiated in 0.5% ammonium hydroxide. After differentiation, the grey matter was counterstained in Neutral Red then rinsed in dH_2O. Finally, sections were dehydrated and cleared, as above, and then mounted onto silane-coated SuperFrost- Plus slides (Fisher Scientific, Pittsburgh, PA).

Our analysis shows that near the epicenter the amount of tissue damage tend to be increased in the PE group compared to the control animals (Figure 1). In the NE group a similar increase in tissue damage was observed, but to a lesser extent compared to the PE group (Figure 1). Whether the increased extent of tissue damage will account for poorer functional recovery is currently unknown (this question will be further addressed in AIM 1-3 of this grant).

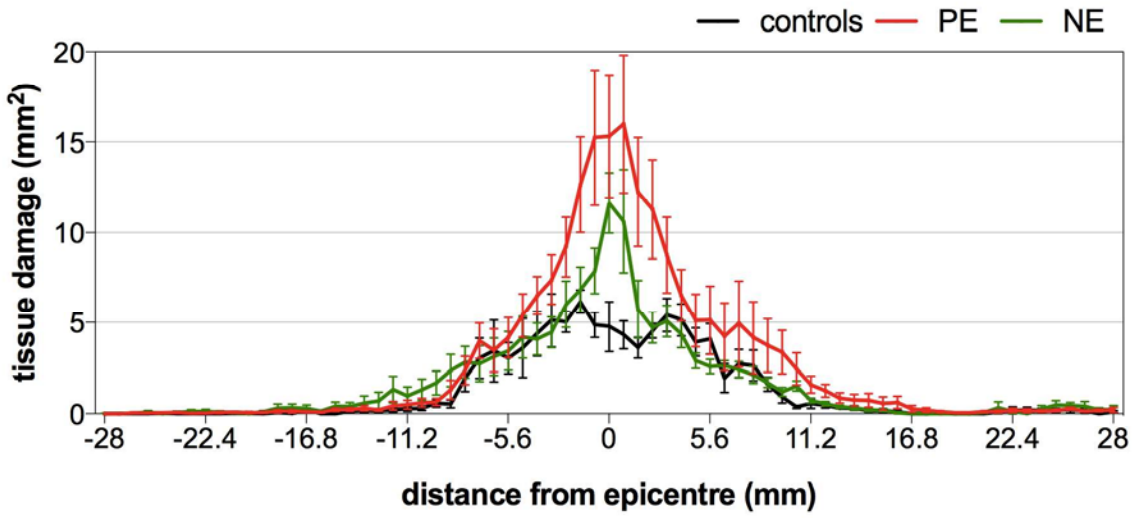
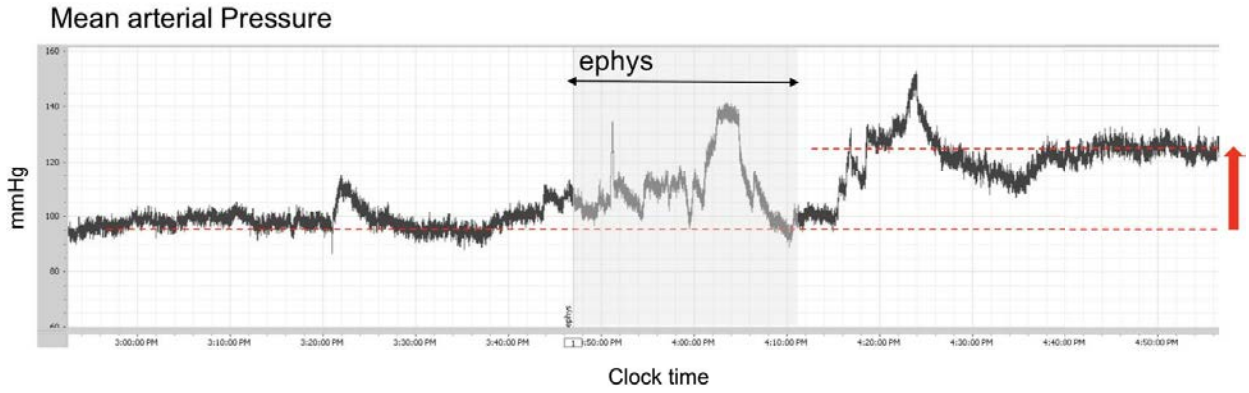


Figure 1: Effect of PE and NE infusion after SCI on total tissue damage. Tissue damage (white and grey area combined) determined by area measurements taken from axial sections of spinal cord tissue 800 μm apart. Near the epicenter ("0") the amount of tissue damage tend to be increased in both the PE and NE group compared to the control animals. Data is expressed as mean ± SEM. NE; norepinephrine; PE: phenylephrine; SCI: spinal cord injury.

In summary, we found that NE was the most 'optimal' vasopressor and provided a better improvement in blood flow and $PaPO_2$ within the injured spinal cord compared to PE. Based on these findings we will use NE in Aim 2 and Aim 3 to determine the effects of aggressive hemodynamic support after SCI on the acute physiologic responses as well as long-term functional outcome.

In our first animals to be studied to address Aim 2 and 3 (animals A and B, Table 3), we were surprised to find that the MAP was quite unstable and in fact, even without vasopressors, often exceeded 100 mm Hg. This certainly affected our ability to move forward with this study, as it was clear that we would be unable to evaluate the effectiveness of MAP augmentation with vasopressors if the animal was naturally this hypertensive post-injury. An example of this is shown below

Figure 3:



Interestingly, this spontaneous increase in MAP is something that we have not previously encountered in past pig surgeries. Further experiments animals D-L, Table 3) suggested to us that this dramatic rise in the MAP was most likely related to the E-phys procedure and the anaesthesia protocol used for this. We could not find precedent for this MAP problem in the E-phys literature using rat or mouse models. e However, based on these observations, we have dropped the E-phys recordings from our study and included 1-0.5% isoflurane to the anesthesia protocol. We continued with the scheduled surgeries as outline in Table 3.

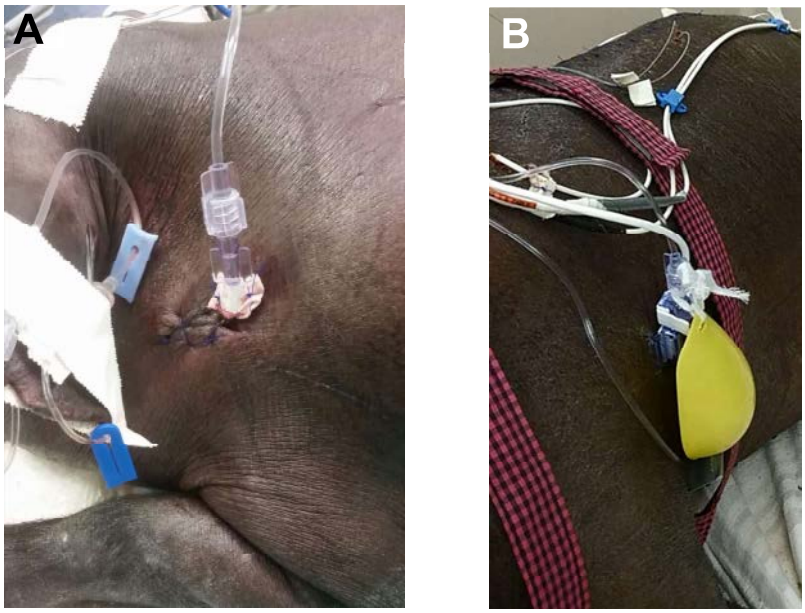
Table 3. Experimental information

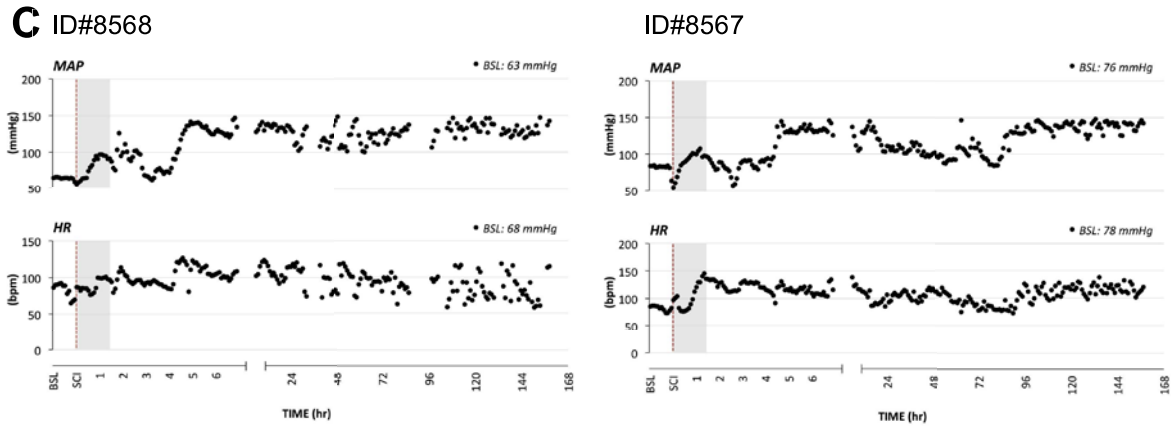
ID	Name	Species	SX date	Injury Specifics	Force	Weight (kg)	Treatment	Notes
8178	Andrew the Giant	Yucatan	1-Apr-16	**Contusion:** 50gr/20cm **Compression:** 150gr/1.5hr	2585.52	25.0	CNTR	**PILOTTING: unstable MAP** **MAP infusion:** 60-min Norepinephrine 20 mmHg above BSL **Groups:** - CNTR: no infusion - NE_{comp}: NE during compression only - NE_{decomp}: NE during decompression only - NE-NE: NE throughout **Anesthesia:** A-E: propofol, fentanyl, 30-40% O_2 F-onwards: isoflurane, propofol, fentanyl, 30-40% O_2 **Ephys:** Animal A-E only
8177	Brett Hart	Yucatan	6-Apr-16		3210.77	26.0	CNTR	
8202	Coco B. Ware	Yucatan	24-Apr-16		2967.00	26.5	$NE_{comp\ (30\ min)}$	
8192	Diamond D. Page	Yucatan	27-Apr-16		3609.00	25.0	CNTR	
8179	Edge	Yucatan	3-May-16		3423.00	26.0	CNTR	
8208	Fabulous Moolah	Yucatan	5-May-16		3438.00	26.0	CNTR	
8275	Golddust	Yucatan	16-May-16		3086.00	26.6	CNTR (†<24hrs)	
8291	Hulk Hogan	Yucatan	18-May-16		2703.00	24.0	$NE\text{-}_{decomp}$	
8278	Iron Sheik	Yucatan	30-May-16		3812.00	28.4	$NE\text{-}_{comp}$	
8303	Jake the Snake	Yucatan	1-Jun-16		3319.00	28.5	$NE\text{-}_{comp}$	
8308	King Kong Bundy	Yucatan	7-Jun-16		3438.00	29.5	$NE\text{-}_{decomp}$	
8310	Legion of Doom	Yucatan	9-Jun-16		3090.00	25.5	NE-NE (†<48hrs)	
8568	Macho Man	Yucatan	03-Oct-16	**Contusion:** 50gr/20cm **Compression:** 150gr/1.5hr	3144.0	25.5	NE-NE	NO EPHYS included because of increasing MAP after MEP/SSEP session **MAP infusion:** 60-min Norepinephrine 20 mmHg above BSL Anesthesia: 1-0.5% isoflurane, propofol, fentanyl, 30-40% O_2
8567	Natalya	Yucatan	05-Oct-16		3274.00	25.5	NE-NE	
	Owen Hart	Yucatan	17-Oct-16					
	Primo	Yucatan	19-Oct-16				†<24hrs)	
			25-Oct-16					
			27-Oct-16					
			21-Nov-16					
			23-Nov-16					
			29-Nov-16					
			01-Dec-16					
			12-Dec-16					
			14-Dec-16					

***Notably, in our award we proposed 3 hours of compression and another 3 hours of decompression with or without MAP support of 20 mm Hg. However, by the end of this 6-hour period, the animal has been under anaesthesia for close to 18 hours, has undergone an extensive laminectomy, and has lost a considerable amount of blood. Hence, we intend 1 hour of compression and another 1 hours of decompression instead, to reduce the risk for serious health complications when animals recovering from anesthesia.

Besides measuring spinal cord pressure, oxygenation and blood flow, we have now also established the technique to accurately monitor MAP changes over a 7-day period. To accomplish this, we tunnelled the arterial catheter underneath the skin (Figure 2A) to avoid abscess and infection at the incision site. Additionally, to ensure accuracy, the pressure transducer was sutured at the level of the right atrium instead of attaching it to the moving harness, and is now covered with a stable protector (Figure 2B - yellow).

Figure 2: (A) Tunnelling of the artery pressure line from the jugular incision to the back of the ear. **(B)** The pressure transducer measuring MAP is sutured at the level of the heart directly to the pig to avoid movement affecting MAP readings and covered with a yellow protector. (C) Mean arterial pressure and heart rate data recorded over a 7-day period of pig ID# 8568 (left) and ID#8567 (right). Grey shading: represents NE infusion.





In summary, we have performed 4 additional surgeries (Table 3: M-P; highlighted in blue). Additional surgeries are scheduled up to December of this year. We will continue with the rest of the animals in the four infusion groups (n=12) and will finish these surgeries by the end of 2016.

5 KEY RESEARCH ACCOMPLISHMENTS

- Proximal to the impact, NE and PE resulted in only a modest improvement in SCBF during cord compression; however, levels remained well below pre-injury values.
- During the decompressed state, NE resulted in increased SCBF and $PaPO_2$ levels, while a decrease was observed for PE.
- Distal to the impact, both vasopressors increased SCBF and $PaPO_2$ above pre-injury levels, with minimal apparent effect on the downstream metabolic state of the cells.
- SCBF continued to rise in the PE group even after infusion was ceased.
- Tissue damage tend to be increased in the PE group compared to the control animals
- In the NE group a similar increase in tissue damage was observed, but to a lesser extent compared to the PE group

6 CONCLUSION

This research focuses on the acute hemodynamic management of spinal cord injury and specifically addresses the current practice of aggressively maintaining the mean arterial pressure (MAP) at 85-90 mm Hg for 5-7 days post-injury. This has particular relevance to military personnel who are injured in combat zones. Our data advanced our current understanding of the pathophysiology following spinal cord injury, and in Year 3 the focus of this study is to finalize the cohort of animals evaluating the consequences of hemodynamic support on intraparenchymal SCBF, oxygenation and downstream metabolic responses after SCI as well as functional recovery.

7 PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

Poster presentation, Society for Neuroscience 2016, Chicago, Illinois, Nov 12-16:

OXYGENATION, PRESSURE AND LONG-TERM BEHAVIORAL RECOVERY USING A PORCINE MODEL OF SCI.

A. GHEORGHE[1], F. STREIJGER[1], K. SO[1], E. B. OKON[1], N. MANOUCHEHRI[1], K. SHORTT[1], D. E. GRIESDALE[2], M. S. SEKHON[3], B. K. KWON[4]

[1]ICORD (International Collaboration on Repair Discoveries), Univ. of British Columbia, Vancouver, BC, Canada; [2]Vancouver Gen. Hospital, Div. of Critical Care Med., Dept. of Anesthesiology, UBC, Vancouver, BC, Canada; [3]Vancouver Gen. Hospital, Div. of Critical Care Med., Dept. of Medicine, UBC, Vancouver, BC, Canada; [4]Vancouver Spine Surgery Inst., Dept. of Orthopaedics, UBC, Vancouver, BC, Canada

Abstract: Traumatic spinal cord injury (SCI) results in local and systemic vascular changes, which make the spinal cord extremely vulnerable to ischemia, hypoxia, and energy dysfunction. Current guidelines recommend maintaining a mean arterial pressure (MAP) of 85-90 mmHg using volume expansion agents and vasopressors in an attempt to support the injured spinal cord with adequate perfusion. While the desire to prevent ischemia is understandable, indiscriminate augmentation of MAP in a traumatically injured cord with impaired vascular autoregulation may have deleterious effects. In this study we sought to determine the impact of MAP augmentation on vascular and metabolic responses as well as long-term behavioral outcomes following a contusion-compression injury of the spinal cord.

Using our porcine model of SCI, female Yucatan miniature pigs received a T10 contusion injury followed by 3-hours of sustained compression. 1-hour post-compression and decompression, norepinephrine (NE) was used to elevate MAP by 20 mmHg for a 1.0-hr period. Laser Doppler/oxygenation and pressure probes were inserted into the spinal cord 0.2 and 2.2 cm from the injury site to monitor the effects of MAP support on spinal cord blood flow (SCBF), PaPO2, pressure over a 7-day period. Microdialysis samples were also collected and subsequently analyzed for lactate, pyruvate, glucose, glutamate and glycerol. Behavioral recovery was scored weekly according to the Porcine Thoracic Injury Behavioral Scale (PTIBS). Data from the first 6 hours after SCI showed that proximal to the impact (0.2-cm location), NE infusion during the compressed and decompressed state of the spinal cord resulted in only a slight restoration of SCBF and PaO2, though both still remained well below pre-injury levels. In the decompressed state, a decrease in L/P ratio was observed in the NE group to ~250% of pre-injury levels, while levels remained unaltered in the control group (~800% of pre-injury). Distal to the impact (2.2-cm location), NE infusion increased SCBF and

PaPO2 above pre-injury levels both during the compressed and decompressed state of the spinal cord. Currently we are analyzing the intraparenchymal responses during the subsequent 7 days as well as the long-term behavioral consequences of MAP support.

Combined, our preliminary results suggest that MAP augmentation during compression and following decompression could partially restore post-traumatic ischemia/hypoxia at the injury site, but could potentially lead to hyperemia distal to the injury site. Identifying the functional consequences is currently being carried out.

Session Type: Poster
Session Number: 323
Session Title: Spinal Cord Injury Models and Mechanisms
Date and Time: Monday Nov 14, 2016 8:00 AM - 12:00 PM
Location: San Diego Convention Center: Halls B-H
Abstract Control Number: 15628

8 INVENTIONS, PATENTS AND LICENSES

Nothing to report

9 REPORTABLE OUTCOMES

Nothing to report

10 OTHER ACHIEVEMENTS

Nothing to report

11 REFERENCES

Nothing to report

12 APPENDICES

Appendix 1 – Draft manuscript in preparation for J Neurotrauma (see Page 18)

13 FINANCIAL HEALTH

FMS GL Summary - Extracted on 22-OCT-2016 02:35 PM
Report Parameters: Fund [R4300] Dept [17700D] Program [] Project [17R21838]
Date Range: From NOV-2015 to OCT-2016
PG Year End: SEP

Description	YTD	Nov-15	Dec-15	Jan-16	Feb-16	Mar-16	Apr-16	May-16	Jun-16	Jul-16	Aug-16	Sep-16	Oct-16	Period Total	PG YTD	YTD
Net Assets-Research-Restricted	-247,018.31	0	0	0	0	0	0	0	0	0	0	0	0	**0**	-267,942.58	0
Sub Total	-247,018.31	0	0	0	0	0	0	0	0	0	0	0	0	**0**	-267,942.58	0
Grants-US government	0	0	0	0	-202,864.99	0	0	0	0	0	-189,034.40	0	0	**-391,899.39**	0	0
Sales-Services	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	0	0
Sub Total	0	0	0	0	-202865	0	0	0	0	0	-189034.4	0	0	**-391,899.39**	0	0
Budget Balance Carry Forward	644,600.36	0	0	0	0	0	0	0	0	0	0	0	0	**0**	0	0
Budget pool-Expense	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	0	0
Salaries-Students(Instruc&Res)	0	1,750.00	1,750.00	1,750.00	1,750.00	1,750.00	1,750.00	1,750.00	1,750.00	-4,492.02	1,750.00	-22,042.54	0	**-10,784.56**	0	0
Salaries-Staff	0	3,914.00	4,033.20	3,953.14	3,953.14	3,953.14	3,953.14	3,970.94	3,970.94	3,990.78	3,990.78	3,369.50	0	**43,052.70**	0	0
Employee benefits-Main	0	413.59	423.39	421.78	656.01	539.03	538.9	871.21	871.2	874.45	874.45	255.16	0	**6,739.17**	0	0
Employee benefits-insurance	0	27.4	27.68	27.68	27.68	27.68	27.68	27.8	27.8	27.94	27.94	23.58	0	**300.86**	0	0
Couriers	0	0	0	0	0	155.66	0	0	0	426.31	0	0	0	**581.97**	0	0
Customs & freight	0	0	0	97.63	228.12	40.46	119.58	679.36	26.3	164.85	143.75	180.91	182.54	**1,863.50**	182.54	0
Creative services-Print	0	0	0	0	0	103.22	0	0	0	0	0	0	0	**103.22**	0	0
Technical supplies	0	124.05	2,854.41	2,229.53	5,401.14	1,183.17	3,677.89	26,685.37	3,724.12	2,706.79	7,484.67	6,848.64	0	**62,919.78**	0	0
Technical services	0	0	0	0	0	0	0	86.58	0	0	0	0	0	**86.58**	0	0
Laboratory supplies	0	0	0	0	0	0	0	181.1	241.83	49.94	0	0	0	**472.87**	0	0
Animal costs	0	90	7,694.90	0	90	19,344.55	0	49,288.04	48,188.14	32,874.14	6,234.50	2,397.86	16,181.22	**182,383.35**	16,181.22	0
Bank charges	0	0	0	0	15.21	0	0	0	8.99	0	9.22	9.18	0	**42.6**	0	0
Research	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	0	0
Maintenance-Equipment	0	189.05	0	0	0	0	0	0	0	0	0	0	0	**189.05**	0	0
Repairs	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	0	-4,896.75
Rentals-Other	0	0	0	0	0	0	0	0	0	0	8,280.00	0	0	**8,280.00**	0	0
Rentals-Equipment	0	0	0	0	0	0	0	0	10,556.32	0	9,864.33	0	0	**20,420.65**	0	0
Overhead	0	3,321.12	3,391.44	3,347.01	3,474.44	3,410.80	3,410.72	3,601.25	3,601.24	218.23	3,613.88	-10,006.51	0	**21,383.62**	0	0
Equipment	0	0	0	0	0	16,915.73	0	2,025.68	0	13,998.35	0	0	0	**32,939.76**	0	0
Sub Total	644,600.36	9,829.21	20,175.02	11,826.77	15,595.74	47,423.44	13,477.91	89,167.33	72,966.88	50,839.76	42,273.52	-18,964.22	16,363.76	**370,975.12**	16,363.76	-4,896.75
Total Expenses:	644,600.36	9,829.21	20,175.02	11,826.77	15,595.74	47,423.44	13,477.91	89,167.33	72,966.88	50,839.76	42,273.52	-18,964.22	16,363.76	**370,975.12**	16,363.76	-4,896.75
														Balance Available:	**633,133.35**	

Appendix 1 – Draft manuscript in preparation for J Neurotrauma

Differing Effects When Using Phenylephrine and Norepinephrine to Augment Cerebral Blood Flow After Traumatic Spinal Cord Injury in Pigs

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Key words: Spinal Cord Injury, Porcine model, Vasopressor Support, MAP, Microdialysis, Blood Flow, Oxygenation, Compression

Abstract

Surgeons elevate mean arterial blood pressure (MAP) to support spinal cord perfusion in various settings, including acute traumatic spinal cord injury (SCI). Norepinephrine (NE) and phenylephrine (PE) are commonly used to elevate MAP in this context, although they differ in their basic pharmacologic properties and potentially have different effects on spinal cord blood flow and oxygenation after injury. Using a porcine model of SCI we evaluated how these two vasopressors influenced spinal cord blood flow (SCBF), oxygenation ($PaPO_2$), pressure, and metabolism.

Yucatan minipigs underwent a contusion SCI at T10 and were randomized to either NE or PE for MAP elevation. Prior to injury, a combined SCBF/tPO2 sensor, a pressure sensor, and a microdialysis probe were inserted into the spinal cord adjacent to T10. SCBF, tPO2, pressure, and metabolic responses were monitored for 3 hours during sustained compression and then 3 hours post-decompression. During each 3-hour period, NE or PE was used to elevate MAP by 20 mm Hg for 1 hour to measure responses inside the injured cord.

Proximal to the impact, NE and PE resulted in a modest improvement in SCBF during cord compression; however, levels remained well below pre-injury values. During the decompressed state, NE resulted in increased SCBF and $PaPO_2$ levels, while a decrease was observed for PE. After decompression, both NE and PE demonstrated a gradual drop in L/P ratio. Distal to the impact, both vasopressors increased SCBF and $PaPO_2$ above pre-injury levels, with minimal apparent effect on the downstream metabolic state of the cells. Notably, SCBF continued to rise in the PE group even after infusion was ceased.

Combined, our results suggest that NE promotes better restoration of blood flow and tissue oxygenation than PE. Given that physicians treating acute SCI patients have their choice of vasopressors to use, there may be a physiologic rationale for selecting NE over PE.

Introduction

Sadly, there are currently very few treatments to improve the neurologic outcome of individuals who sustain an acute spinal cord injury (SCI). Treatment options include urgent surgical decompression to relieve pressure on the spinal cord and aggressive augmentation of systemic blood pressure to minimize ischemia. Despite considerable preclinical evidence that hypoperfusion and ischemia are significant contributors to secondary damage after traumatic SCI,[1,2] translating this knowledge to the clinical care of acute SCI patients has not been straight-forward. Early non-randomized clinical studies reported that elevating mean arterial pressure (MAP) to 85-90 mmHg for 5-7 days via aggressive fluid and vasopressor support improved mortality rates and neurological outcome after acute SCI.[3,4] Based on this, many have adopted the clinical practice of using vasopressors and/or intravenous fluids to augment the MAP to 85-90 mmHg for the first 7 days post-injury in acute SCI. The hemodynamic management of acute SCI is currently one of the few aspects of clinical care where physicians can potentially improve neurologic outcome. Thus, there is a strong practical rationale to better understand the hemodynamic management of acute SCI and optimize treatment guidelines – both in defining the optimal MAP targets and how to best achieve them with which vasopressors.

The ultimate goal for the hemodynamic management of acute SCI is to optimize neurologic function by restoring adequate perfusion to meet the metabolic needs of the injured cord. Current guidelines for acute SCI suggest a potential benefit from augmenting MAP to 85-90 mmHg with intravenous fluids and vasopressors. A number of different vasopressors are used clinically to support MAP, including phenylephrine (PE), dopamine (DA) and norepinephrine (NE).[5] There are, however, no formal recommendations regarding the optimal agent in acute SCI. The decision about which vasopressor to use in acute neurotrauma is typically left to the discretion of the treating physician, whose choice may be based on desired pharmacologic activity, personal familiarity/experience, and institutional bias/tradition. This has resulted in considerable variations in clinical practice for both traumatic brain injury (TBI) and SCI. For example, a retrospective study of pediatric TBI patients at a single American level 1 trauma center reported that vasopressor usage varied widely, with the first-line treatment most commonly being PE (57%), followed by DA (29%), NE (10%), and epinephrine (4%).[6] In a series of 114 adult TBI patients

from the same institution, PE was again the most commonly used vasopressor (43%), followed by NE (30%), DA (22%), and vasopressin (5%).[7] In a series of 131 acute SCI patients at another American level 1 trauma center, DA was the most commonly used vasopressor (48%), followed by PE (45%), NE (5.0%), epinephrine (1.5%) and vasopressin (0.5%).[8] In contrast, at our local institution and in other Canadian institutions, the vasopressor of choice is NE, followed by DA. These observed variations in practice simply highlight the equipoise that exists with respect to vasopressor usage in acute SCI and TBI.

While all of these vasopressors can effectively increase MAP, they each have unique pharmacologic properties, based on their affinity for alpha-adrenergic, beta-adrenergic, dopamine, and vasopressin receptors. As such, it is reasonable to expect that they would have different effects on perfusion within the injured central nervous system (CNS).[9] NE is a potent alpha-adrenergic agonist with less pronounced beta-adrenergic agonist effects. NE increases MAP primarily by peripheral vasoconstriction but also potentially increasing cardiac output through beta1-mediated inotropy. DA has dose-dependent effects, with dopaminergic effects at low doses and mixed alpha- and beta-adrenergic effects at higher doses, causing vasoconstriction and increased cardiac output. PE, a selective alpha1-adrenergic agonist, increases MAP primarily by peripheral vasoconstriction. Its rapid onset, short duration, and primary vascular effects make it an attractive agent in the management of hypotension. However, because of its isolated alpha1 effects, PE can lead to reflex bradycardia and decreased cardiac output, leading to worsened tissue perfusion.[10] Although these well-described profiles characterize the overall cardiovascular effects of vasopressors, their effects on specific vascular beds within the CNS may not be so predictable, particularly after injury.[11,12] Thus, understanding how commonly used vasopressor-inotropes affect vascular mechanisms within the spinal cord is of major clinical relevance, particularly in the setting of traumatic SCI.

Therefore in this study we performed a head-to-head comparison of NE, and PE after SCI to determine how these commonly used vasopressors affects spinal cord oxygenation (luminescence-based optical

sensor), perfusion (laser-Doppler flowmetry), intraparenchymal pressure (fiber optic probe) and downstream metabolic responses (microdialysis). Recognizing the translational importance of the pig,[13-15] for this study we used female Yorkshire pigs that were subjected to a combination of contusion trauma and compression at the T10 level.[16]

Methods

All animal protocols and procedures employed in this study were approved by the Animal Care Committee of the University of British Columbia and were compliant with the policies of the Canadian Council on Animal Care.

Animals & experimental grouping

Female Yorkshire pigs (local distributor) weighting 25-32 kg were transported to our animal facility 1 week before surgery. Upon arrival, the animals were housed in groups of 2-4 in an indoor pen bedded with sawdust and toys (chains, balls) with access to an adjoining outdoor pen. Animals were given water *ad libitum* and fed 1.5% of their body weight twice a day (Mazuri Mini-Pig Youth, PMI Nutrition International, Brentwood MO, USA). On the day of surgery, animals were distributed into three groups: 1) SCI-NE group, n=9 2) SCI-PE group, n=9, and 3) SCI-controls, n=4. All three groups received a T10 contusion injury followed by 3 hours of compression and then 3 hours of post-decompression. During each 3-hour period, the SCI-NE and SCI-PE group received a 1-hour infusion of NE (4 mg in 250 ml of 0.9% saline/1.25% dextrose) or PE (1ml in 250 ml of 0.9% saline/1.25% dextrose) respectively to raise MAP 20 mmHg and measure responses inside the injured cord. The SCI-control group received no vasopressor infusion. During vasopressor infusion, I.V. maintenance fluids rates (0.9% NaC/1.25% dextrose) were independently adjusted to ensure a total fluid infusion of 7ml/kg/hr for all three experimental groups.

Catheterization of the carotid artery and jugular vein

Tracheal intubation and mechanical ventilation was performed as described previously.[17] Anesthesia was maintained with a combination of isoflurane (0.5% in 100% O_2) and propofol (8-20 mg/kg/hr; Baxter, Allison, Ontario, Canada). All animals received ketoprofen (3 mg/kg) via intravenous administration and fentanyl (15-30 g/kg/hr; Sandoz Canada, Boucherville, Quebec, Canada) delivered via continuous rate infusion (CRI). After a surgical plane of anesthesia was reached, the animal was placed on the surgical table in the supine position. Using a scalpel, a longitudinal incision was made ~3 cm left of the midline. After the perivascular connecting tissues were carefully removed by blunt dissection, catheter was inserted into the left external jugular vein (EJV) for the application of drugs and into the common carotid

artery (CCA) for the measurement of MAP. After exposure of the CCA, a 14 gauge i.v. catheter was inserted, which was then connected to a blood pressure transducer system (Edward Lifesciences Inc, Irvine, CA). After the EJV was ligated, a 7F triple lumen venous catheter (Arrow International, Reading, PA) was inserted and advanced ~16 cm towards the heart. To secure both catheters, two ligatures caudal to the site of insertion were carefully tied around the vessels in addition to being sutured to the musculature. The wound was closed in layers with the catheters exiting the skin through the primary incision site.

Porcine model of SCI

After catheter insertion, the animal was rotated into a prone position. A skin incision was made along the dorsal midline of the thoracic region of each animal. Using electro-cautery (Surgitron® Dual Frequency RF/120 Device; Ellman International, Oceanside, NY), the semispinalis, multifidus and longissimus lumborum muscles were separated from the dorsal spinous processes, laminae and transverse processes. A T9 to T13 laminectomy was performed and widened to expose the dura and spinal cord with sufficient clearance for sensor insertion and weight drop injury.

The SCI was delivered by a weight drop impactor device, which was securely fixed to the spine using an articulating arm (660, Starrett, Athol, Massachusetts, USA) mounted via bilaterally inserted T6 and T8 pedicle screws. This arm enabled the guide-rail to be precisely positioned and aligned, allowing for the impactor to fall straight vertically onto the exposed dura and cord at T10. The tip of the impactor (diameter: 0.953 cm) was outfitted with a load cell (LLB215, Futek Advanced Sensor Technology, Irvine, CA, USA) to record the force at impact. The guide rail was equipped with a Balluff Micropulse® linear position sensor (BTL6-G500-M0102-PF-S115, Balluff Canada Inc., Mississauga ON, Canada) to record the impactor position from 10 cm above the impact (for calculation of impact velocity and cord displacement). A custom controller was used to operate the device and filter the force and position data collected with the simultaneous USB DAQ module (DT9816-S, Data Translation Inc., Marlboro, MA, USA). A LabVIEW (National Instruments, Austin, TX, USA) program enabled remote operation of the device and real-time data collection feedback. Immediately after the weight drop contusion injury (weight:

50 g; height: 20 cm), a 3-hour compression period was maintained on the contused spinal cord by placing an additional 100 g weight onto the impactor (150 g total).

Insertion of the intraparenchymal blood flow/O_2, pressure, and microdialysis probes

To consistently insert the monitoring probes into a desired location within the spinal cord and hold them there for the duration of the experiment, a custom-made sensor holder was created. The sensor holder was attached rigidly to the spine via bilateral T9, T11, T12, and T14 pedicle screws (Select ™ Multi Axial Screw, Medtronic, Minneapolis, MN) and 3.5 mm titanium rods (Medtronic, Minneapolis, MN). The location of the sensor holder was locked in place by sliding the device over the rod and adjusting the height of the pedicle screws. Additional transverse connecting bridges were fixed between two poly-axial pedicle screws to produce a stable, rigid construct. Six custom-made introducers with a lumen wide enough to fit one sensor/probe were inserted through precision-drilled holes in the sensor-holder (45-degree angle), entering the dura at 1.2 and 3.2 cm from the centre of the intended impact. Subsequently, the sensors were guided through the introducers and advanced another 0.78 cm, placing the sensors in the ventral aspects of the white matter. The distance between adjacent tip locations was 0.5-1.0 mm. The final location of the tip of the sensors were situated 2 mm and 22 mm away from the edge of the impactor. Ultrasound imagining (L14-5/38, 38 mm linear array probe, Ultrasonix RP, BK Ultrasound, Richmond, BC, Canada) was utilized to verify accurate positioning of sensors and probes into the cord. To prevent CSF leakage, cyanoacrylate glue was applied to the dural surface where the catheters entered. After sensor insertion, we waited for 2 hours to allow for a period of 'stabilization'. We then commenced recording the 'baseline' samples for a period of 60 minutes prior to the actual spinal cord injury.

Measure of intraparenchymal blood flow and partial pressure of oxygen ($PaPO_2$)

For measurement of blood flow and oxygen, we utilized a single multi-parameter probe that contains at the tip a device for measuring flow and another for measuring $PaPO_2$. This probe, with a tip diameter of 450 m (NX-BF/OF/E, Oxford Optronix, Oxford, UK), was attached to the OxyLab/OxyFlo combined channel monitor (Oxford Optronix OxyLab, Oxford, UK) with LabChart Pro software for interpretation (ADInstruments, Colorado Springs, Colorado, US).

Tissue oxygen partial pressure ($PaPO_2$) was measured by a fluorescence quenching technique which monitors the mean time between photon absorption and emission of the oxygen-sensitive fluorescent ruthenium lumiphor dye after being excited by a short pulse of light (luminescence lifetime). In the presence of oxygen, the fluorescence lifetime is quenched proportionally to the oxygen concentration. The OxyLab system measures the reduced lifetime of luminescence of the reflected signal and displays a value for partial pressure of oxygen ($PaPO_2$) in mmHg according to the Stern Volmer equation. Since the luminescence-based oxygen sensing technique is sensitive to temperature changes, a thermocouple transducer is incorporated into the sensor for temperature corrections for variations from 30-44^{o}C. Blood flow is determined via laser-Doppler flowmetry where light from the probe tip is projected into the tissue, scattered and then reabsorbed by a sensor. Only the laser light backscattered from moving cells undergoes a Doppler shift, which creates Doppler frequencies at the detectors and produces a voltage output which can be interpreted as blood flow by the OxyFlo monitor (in arbitrary perfusion units, APU). The numeric calculation of LDF is dependent on the relative concentration of local red blood cells in the tissue and the velocity.

Intraparenchymal pressure measurement

Spinal cord pressure was characterized using custom-manufactured fiber optic pressure transducers (FOP-LS-NS-1006A, FISO Technologies Inc., Harvard Apparatus, Quebec, Canada) with a sensor tip diameter of 333 m. These transducers operate based on Fabry-Perot interfermometry. The sensor tip is comprised of two parallel reflecting mirrors on either side of an optical cavity. The first mirror is semi-reflective and the second is a flexible membrane. As pressure is applied, the membrane deflects, reducing the cavity length. The reduced cavity lengths cause phase shifts in the reflected light, which are distinguished by a detector. Transducers are calibrated in such way that each cavity length corresponds to a specific pressure value, with the transducers being capable of measuring pressure changes of ± 300 mmHg, with a resolution of ± 0.3 mmHg. Transducers were connected to a chassis-mounted signal

conditioner module (EVO-SD-5/FPI-LS-10, FISO Technologies Inc., Harvard Apparatus, Quebec, Canada) with internal atmospheric pressure compensation, which is particularly valuable for long-term animal studies. The data was acquired digitally through the Evolution software (FISO Technologies Inc., Harvard Apparatus, Quebec, Canada) at a frequency of 1 Hz.

Microdialysis

Microdialysis probes (CMA11, CMA Microdialysis, Harvard Apparatus, Quebec, Canada) with an outer diameter of 380 m, 2 mm membrane length and a 6-kDa cutoff were used to sample the extracellular fluid for energy related metabolites. Probes were continuously perfused with artificial CSF (Perfusion Fluid CNS, CMA Microdialysis, Harvard Apparatus, Quebec, Canada) using a subcutaneous implantable micro-pump at a flow rate of 0.5 ul/min (SMP-200, IPrecio, Alzet Osmotic Pumps, Durect Corporation, Cupertino, CA, USA). Dialysates were collected in micro tubes, capped, and frozen on dry ice every 15 minutes, from the beginning of the baseline period to 6 hours post-injury; providing a sample volume of 7.5 µl sufficient for the exploration of five metabolites (lactate, pyruvate, glucose, glutamate and glycerol). Samples were analyzed within a week of collection using the ISCUS^{flex} Microdialysis Analyzer (M Dialysis, Stockholm, Sweden).

Data analysis

SCBF and $PaPO_2$ were recorded continuously at a sampling rate of 10 Hz during baseline recording and for 3 hours after SCI. Thereafter the sampling rate was reduced to 1/6 Hz, as the sensor carries only a limited supply of the ruthenium lumiphor. To mitigate movement artifacts in the oxygenation and blood flow data, a post-processing filter was applied (smoothing type: median filter, window width: 601 samples / 1 minute of sampling). Microdialysis samples were collected every 15 minutes, from the beginning of the baseline period to 6 hours post-injury. These duplicate values were averaged for each time point. The delay from microdialysis catheter tip to collecting vial was 12 minutes, and this was accounted for when analyzing the results. The lactate to pyruvate (L/P) ratio was calculated from the measured values of lactate and pyruvate concentrations. To account for absolute differences in the baseline recordings, all values were expressed as a percentage change from baseline (%Δ) as a function of time (hours). For

comparisons of three independent groups, the Kruskal-Wallis test followed by Dunn's post hoc test was used. Between group differences at each time point was determined using multiple t-test. Calculated p-values were corrected for multiple testing using Bonferroni adjustment. Differences between groups were considered significant if more then two consecutive points in the time course were statistically significant at $p < 0.05$. Results are expressed as mean values ±SEM, with correlations between parameters determined using Spearman correlation coefficients with Bonferroni correction (GraphPad Prism 6).

Results

Validation of the impact across the experimental groups

All animals received a contusion SCI by dropping a 50 g weight from a 20 cm height at the T10 level of the spinal cord followed by 3 hours of compression (150 g total weight). To determine the consistency of the injury, we measured force, displacement and velocity of the impact. On average the maximum impact force applied to the exposed spinal cord measured at the tip of the impactor was 3315 ± 119 kdynes. The impactor tip traveled 3.1 ± 0.1 mm from initial contact with the exposed dura with a velocity of 1,701 ± 1306 mm/sec at impact. Between the experimental groups no significant differences were observed ($p>0.05$)(Table 1).

Physiological parameters

To maintain a target MAP of 20 mmHg above pre-SCI levels (i.e. target MAP: of 75-85 mmHg; Figure 1A), the average PE dose was 0.7 + 0.1 (range 0.14–2.8) mcg/kg/min, and NE dose was 0.1 + 0.04 (range 0.02–1.18) mcg/kg/min. No differences in target MAP between the SCI-NE and SCI-PE group was observed during the compressed or decompressed state of the spinal cord. Raising MAP with NE by 20 mmHg significantly increased heart rate (HR) over time, while PE showed a tendency to decrease HR (Figure 1B).

Proximal to impact (2-mm probe position)

Post-injury Changes in Intraparenchymal Pressure, SCBF, $PaPO_2$ and Pressure: SCBF and $PaPO_2$ adjacent to the injury site plummeted following SCI indicative of ischemia (Figure 2A-B). During 3-hours of sustained compression, no recovery of blood flow or $PaPO_2$ was observed in the SCI-control group. Both the PE and NE vasopressor groups demonstrated a partial recovery of SCBF during MAP increase of 20 mmHg, although this was less in the SCI-PE group compared to SCI-NE group. Vasopressor infusion had minimal effect on $PaPO_2$ during the compressed state, independent on which vasopressor was used. Following decompression, SCBF and $PaPO_2$ levels recovered partially for all three groups, although the extent was much greater in both vasopressor groups. In the decompressed state only NE was able to

increase both SCBF and $PaPO_2$, while PE infusion resulted in a drop of SCBF. Compression of the spinal cord resulted in increased intraparenchymal SCP to 300-600% above pre-injury levels in all three experimental groups, which rapidly declined after decompression. MAP augmentation by 20 mmHg with PE or NE did not seem to effect SCP values.

Post-injury Changes in Microdialysis Markers: Time-dependent changes in microdialysis markers of cellular distress (glutamate and glycerol) and energy metabolism (glucose, lactate, and pyruvate) induced by the injury are summarized in Figure 3. Glucose levels in the SCI-control group rapidly decreased after contusion to <10% of pre-injury values and steadily decreased to almost zero at the end of the 3-hour compression period (Figure 3A). Decompression was associated with a small increase in glucose, yet levels persisted well below pre-injury values (~25%). In contrary to the gradual drop in glucose levels for the SCI-control group during the 3-hours of compression, glucose held steadily at around 20% above pre-injury values during the 1-hour infusion period in both the SCI-PE and SCI-NE group. For the first 45 minutes after vasopressor infusion was ceased, glucose levels tended to be higher in the SCI-NE group compared to the SCI-control animals ($p<0.05$). During the compression period, the glucose response was similar across the 3 groups.

Glutamate levels sharply increased after SCI, reaching peak values of ~3000% within 15 minutes after contusion (Figure 3B). For the SCI-control group, glutamate levels remained >2000% throughout the 6 hour observation period. During the compressed state of the spinal cord, augmenting MAP resulted in negligible changes in glutamate levels in the SCI-PE and SCI-NE group. However, following decompression the SCI-NE group demonstrated a decreasing trend within 45 minutes after decompression (notably 15 minutes before vasopressor infusion). Glutamate decreased to 600% and remained at this level during the 1-hour period following cessation of NE infusion. Compared to the SCI-control group, glutamate values in the SCI-NE group were significantly ($p<0.05$) lower 0.5 hours after decompression and during the second NE infusion. Although the decline in glutamate was numerically more in the SCI-NE than the SCI-PE (mean percent change -1000% vs. -500%), the difference was not statistically significant.

Glycerol levels exhibited a peak value of ~317% within 30 minutes after contusion and remained at this level throughout the compression period (Figure 3C). Immediately after decompression, levels rose again and remained high at ~500-650% till the end of the study. For the SCI-PE and SCI-NE group, the acute change in glycerol as a result of the SCI (i.e. prior to the first vasopressor infusion) was significantly less. Notwithstanding the lower SCI values, PE and NE infusion had no apparent effect on glycerol levels.

In the SCI-control group, elevated lactate (Figure 3D) and decreasing pyruvate levels (Figure 3E) were observed during the compression period, resulting in a marked rise in L/P ratio (Figure 3F). After decompression, both lactate and pyruvate levels steadily increased, but then decreased again. As lactate levels did not change in direct proportion to pyruvate, L/P ratio gradually declined to ~450%. As in the SCI-control group, a similar increase in L/P ratio was observed acutely after SCI and during the 3-hour compression period for both the SCI-PE and SCI-NE group, without significant changes during vasopressor infusion. However, 30 minutes after decompression (i.e. 30 minutes before the second vasopressor infusion) values started to drop down and continued to decline till state levels of 250% were reached (i.e. 30 minutes after initiation of the infusion). For both the SCI-PE and SCI-NE group, the L/P ratio values were significantly lower 1.0 hour after decompression and onwards compared to SCI-control animals. Between the SCI-PE and SCI-NE groups, the response in L/P ratio was not significantly different at any time.

Distal to impact (22-mm probe position)

Post-injury Changes in Intraparenchymal Pressure, Blood Flow, $PaPO_2$ and Pressure: Compared to the 2-mm probe location, slight changes in SCBF, $PaPO_2$, and pressure after SCI were observed at the 2.2-cm position (i.e. the more distal of the two sensor positions) (Figure 4). An increase in SCBF was found in the SCI-control within 15 minutes after SCI and after spinal cord decompression (Figure 4A), while $PaPO_2$ gradually increased over time to 180% above pre-injury values (Figure 4B). Despite the lower injury response after SCI compared to the SCI-control group, augmenting MAP by 20 mmHg using PE or NE during compression and decompression resulted in a further increase in SCBF and $PaPO_2$ levels. After ceasing NE infusion, SCBF and $PaPO_2$ values in the SCI-NE group dropped immediately to pre-infusion levels and showed a declining trend thereafter. Conversely, SCBF and $PaPO_2$ in the SCI-PE group

gradually continued to increase even after infusion was stopped until the end of the experiment. Vasopressor infusion did not have a noticeable effect on intraparenchymal pressure (Figure 4C).

Post-injury Changes in Microdialysis Markers: Glucose, glutamate, glycerol, lactate, pyruvate and L/P ratio patterns after SCI at the 22-mm position were far less pronounced (Figure 5). Between the experimental groups no significant differences were observed ($p>0.05$)

Vasopressor infusion and correlations between hemodynamic, pressure, and metabolic responses

Changes in SCBF at the 0.2-cm position during the first 2 hours after vasopressor infusion was initiated were compared to changes in $PaPO_2$, pressure and microdialysis responses to define relations between these parameters. The Spearman correlation matrix coefficient (r) was used to determine the relationship between selected parameters (11x11 matrix). The Bonferroni correction for alpha with 55 comparisons is $p<0.0009$.

During and after vasopressor infusion while the spinal cord was compressed, changes in MAP in the SCI-NE group correlated only weakly with SCBF ($r=-323$, $p<0.0001$) and $PaPO_2$ ($r=-0.319$, $p<0.0001$), while no correlations were found between these parameters in the SCI-PE group ($p<0.01$). In both groups, SCBF correlated strongly with $PaPO_2$ (SCI-PE: $r=0.897$; SCI-NE: $r=0.776$; $p<0.0001$). Changes in SCP correlated strongly with changes in MAP in both the SCI-PE ($r=0.801$, $p<0.0001$) and the SCI-NE group ($r=0.918$, $p<0.0001$). Changes in MAP, SCBF or $PaPO_2$ did not correlate with changes in any of the microdialysis markers in both the SCI-PE and SCI-NE group.

During and after vasopressor infusion while the spinal cord was decompressed, changes in MAP correlated positively with SCBF ($r=0.946$, $p<0.0001$) and $PaPO_2$ ($r=0.766$, $p<0.0001$) in the SCI-NE group. Furthermore, in the SCI-NE group a strong positive correlation was found between SCBF and $PaPO_2$ ($r=0.767$, $p<0.0001$). Notably, MAP negatively correlated with SCBF ($r=-0.607$, $p<0.0001$) in the SCI-PE group and correlated only weakly with $PaPO_2$ ($r=0.359$, $p<0.0001$). Moreover, SCBF did not correlate with $PaPO_2$ ($p>0.01$) in the SCI-PE group. Modest correlations were found between SCP and HR ($r=0.645$, $p<0.0001$), MAP ($r=-0.555$, $p<0.0001$), and blood flow ($r=0.652$, $p<0.0001$) in the SCI-PE group. Changes in SCP in the SCI-NE group only correlated with changes in HR ($r=0.645$, $p<0.0001$). Similar as observed during the compressed state, changes in MAP, SCBF or $PaPO_2$ did not correlate with changes in any of the microdialysis markers for both the SCI-PE and SCI-NE group.

Conclusion and Discussion

This study we compared the effect of NE, and PE infusion after SCI on spinal cord oxygenation (luminescence-based optical sensor), perfusion (laser-Doppler flowmetry), intraparenchymal pressure (fiber optic probe) and downstream metabolic responses (microdialysis). For this study we used female Yorkshire pigs subjected to a combination of contusion trauma and compression at the T10 level. These two vasopressors were chosen because in the clinical setting of TBI and SCI, both PE and NE were found to be the commonly utilized to augment MAP.[6-8,18]

Using PE or NE, MAP was augmented by 20 mmHg above pre-SCI levels reaching target levels of around 75-85 mmHg. There was no difference in MAP with the use of PE versus NE in this study. Correlation analysis revealed that changes in MAP during NE and PE in the compressed state of the spinal cord were not associated with positive changes in neither SCBF nor $PaPO_2$. Notably during the decompressed state, there was no relation between changes in MAP and SCBF with PE, while an almost linear correlation existed with the use of NE. Additionally, an augmentation of SCBF correlated directly to positive changes in $PaPO_2$ using NE, which was contrary to PE. Notably, glutamate and L/P ratio levels were significantly lower with the use of NE during decompression compared to no infusion, 1 hour after infusion had stopped indicative of reduced glutamate toxicity and ischemia. This is most likely due to the improved perfusion after decopresion as observed with NE. These results suggest that NE may be preferable to PE if vasopressor support is required post SCI to maintain elevated MAPs in accordance with published guidelines.

The general consensus exist that inadequate perfusion and the consequent metabolic changes are the main causes of neurological deterioration in acute neurological conditions such as traumatic spinal cord injury (SCI). Successful management of these conditions depends on early and accurate identification of ischemia and timely treatment. The exact cause of post-traumatic ischemia remains unknown, although it has been postulated that this is due to

immediate and delayed vascular changes both systemically and locally.[1] The systemic effects include loss of the sympathetic vasomotor tone below the level of the injury, decreased peripheral vascular resistance, hypotension and reduced cardiac output. This is a manifestation of the malfunction of the autonomic nervous system, caused by the absence of sympathetic activity, through the loss of supraspinal control and unopposed parasympathetic tone via the intact vagus nerve.[19,20] Mechanical damage to the spinal cord can furthermore lead to local consequences including post-traumatic loss of vasculature, intraparenchymal hemorrhage, vascular vasospasm, failure of microcirculation in both gray and white matter, impaired autoregulation, and vasogenic edema as a consequence of blood-spinal cord barrier breakdown. All these factors summed up together can lead to impeded nutritive tissue perfusion, making the spinal cord extremely vulnerable to ischemia, hypoxia and energy dysfunction, all of which have significant impact on tissue and functional recovery. Therefore, supporting the injured spinal cord with adequate perfusion is a recognized treatment option after SCI to ensure a constant delivery of oxygen and substrates and to remove the waste products of metabolism.[21,22]

Many literature reviews and guidelines have concluded that hypotension should be avoided in acute SCI patients and Current Level II recommendations are to maintain mean arterial blood pressure at 85-90 mmHg for 5-7 days via aggressive fluid and vasopressor support. Nonetheless, there are no recommendations on the most effective vasopressor and thus the choice of vasopressor agent remains largely empirical and variable. Importantly, while both vasopressors can effectively increase MAP, they each have unique pharmacologic properties and their effects on specific vascular beds within the CNS may not be so predictable, particularly after injury. Studies of cerebral blood flow (CBF) and metabolic responses certainly support this notion that different vasopressors have different effects on CNS perfusion after injury.[23] For example, some studies that have compared NE and DA in animal models of TBI have reported better cerebral perfusion around the injury penumbra with NE,[24,25] and the potential for worsened vasogenic edema with the use of DA.[26] In contrast, others have reported better cerebral

perfusion after TBI with DA than NE.[12] In a randomized, cross-over study of TBI patients, NE resulted in more consistent increases in transcranial Doppler cerebral blood flow velocity when compared to DA.[27] An additional clinical TBI study reported that in contrast to DA, NE consistently increased cerebral oxygenation and decreased the regional oxygen extraction fraction and ischemic brain volume in TBI.[28] Interestingly, in a study of healthy individuals by Brassard et al.[29] NE increased MAP in a dose-dependent manner, but negatively affected cerebral oxygenation. For PE, some animal TBI studies have shown it to improve cerebral perfusion and oxygenation,[30,31] although this may be associated with increased intracranial pressure and vasogenic edema.[32,33] However, in a clinical study of TBI where brain oxygenation was monitored with Licox probes, PE increased cerebral perfusion pressure without improved cerebral oxygenation.[34] In the setting of anesthesia-induced hypotension, PE was also found to reduce cerebral oxygenation by 14%,[29,35,36] findings that are also supported by other investigators studying the effects of PE on cerebral oxygenation in other non-traumatic settings.[10,36,37] While these studies suggest that DA and PE have potential disadvantages in the setting of neurotrauma, it is worth noting that these were the two vasopressors of choice in the large series of acute SCI patients reported by Inoue et al.[8] Clearly, there is room for further study on the use of such vasopressors, particularly in the setting of traumatic SCI.

It is well accepted that normal physiological MAP values for conscious healthy pigs reach 96 ± 2 mmHg,[38] which is similar to humans. Acute hypotension is frequently seen in SCI patients with injuries at or above the T6 level, as the sympathetic outflow to splanchnic vascular beds is lost. Unfortunately due to the natural lordotic curvature of the thoracic spine and substantial bleeding during the procedure of exposing the target vertebrae at or above the T6 level, in this study we choose to perform a T10 SCI instead. Although we did not observe SCI-induced hypotension, over the time course of the surgery MAP did plunge below the hypotensive threshold of 70 mmHg (MAP range: 55-60 mmHg) comparable to human SCI patients. Notably, raising MAP by 20 mmHg, as done in this study, re-establishes normotension (75-85 mmHg), not hypertension. While these MAP values are at or below the recommended target of 85-90 mmHg, we recently reported that the recorded MAP in acute SCI patients frequently dropped 20 mmHg

(or more) below target levels.[39] Hence, our proposed MAP support of 20 mmHg indeed mimics the clinical resuscitation goals for the hemodynamic management of acute SCI patients.

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Table 1. Impact parameters across the experimental groups. There were no differences of injury parameters across groups (Dunn multiple comparison test), indicating the contusion injury was consistent throughout the study with regards to force, displacement and velocity ($p>0.05$). Data presented as mean ± SEM.

Parameters	Group 1	Group 2	Group 3
	controls	PE	NE
N	4	9	9
Body weight (Kg)	29.1 ± 2.7	31.1 ± 1.6	29.9 ± 1.3
Max Force (Kdynes)	2737 ± 191	3477 ± 205	3441 ± 177
Displacement (mm)	3.0 ± 0.2	3.2 ± 0.1	3.1 ± 0.1
Impact Velocity (mm/s)	1621 ± 41	1723 ± 18	1717 ± 16

SCI: spinal cord injury; PE: phenylephrine; NE: norepinephrine

Figure 1. Usage of PE and NE after SCI to augment MAP by 20 mmHg. Animals were distributed into three groups: 1) NE group, 2) PE group, and 3) controls. All three groups received a T10 contusion injury followed by 3 hours of compression [0-3 hpi] and then 3 hours of post-decompression [3-6 hpi]. Decompresion is shown by the vertical dashed line. (**A**) During each 3-hour period, the NE and PE group received a 1-hour infusion of NE (4 mg in 250 ml of 0.9% saline/1.25% dextrose) or PE (1 ml in 250 ml of 0.9% saline/1.25 % dextrose) respectively to raise MAP 20 mmHg above pre-SCI levels (i.e. target MAP: of 75-85 mmHg; see grey shading). (**B**) Raising MAP with NE by 20 mmHg significantly increased HR over time, while PE showed a tendency to decrease HR. Data is expressed as mean ± SEM. HPI: hours post-injury; HR: heart rate; MAP: mean arterial pressure; NE; norepinephrine; PE: phenylephrine; SCI: spinal cord injury.

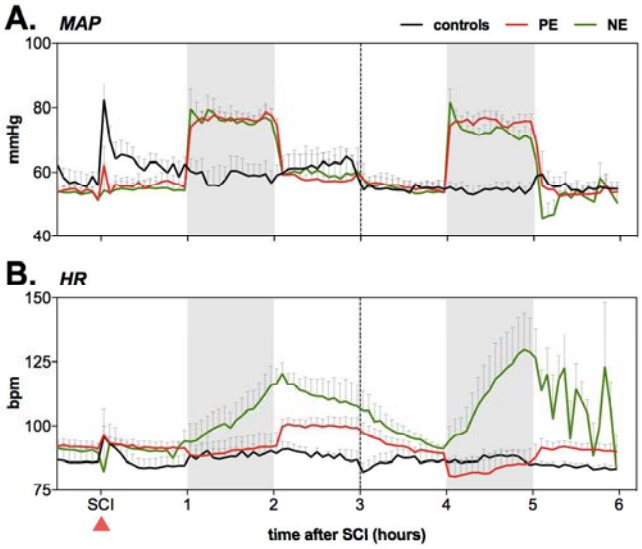

Figure 2. Effect of NE and PE infusion after SCI on blood flow, partial pressure of oxygen and pressure in the penumbra (2-mm). The percentage change (%Δ) is calculated using an average of 30 minutes of baseline before SCI. (**A**) Spinal cord blood flow (SCBF), (**B**) partial pressure of oxygen (PaO_2) and (**C**) pressure responses of 1-hour of vasopressor infusion (grey shading) during the compressed [0-3 hpi] and decompressed [3-6 hpi] state of the spinal cord. Decompresion time is shown by the vertical dashed line. The NE vasopressor group demonstrated a partial recovery of SCBF and $PaPO_2$ during MAP increase of 20 mmHg during compression as well decompression. In the decompressed state PE infusion resulted in a drop of SCBF. Data is expressed as mean ± SEM. HPI: hours post-injury; NE; norepinephrine; PE: phenylephrine; SCI: spinal cord injury.

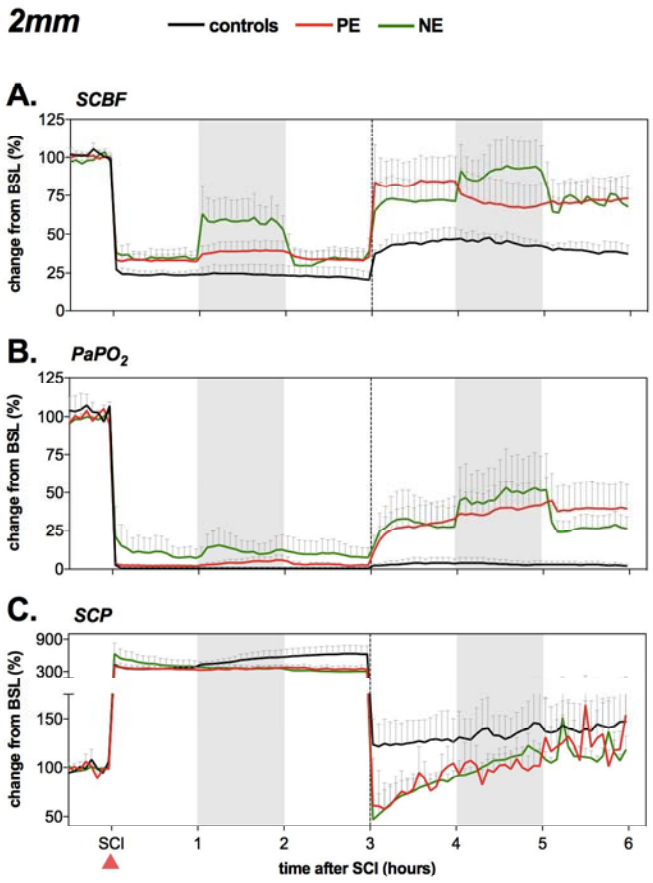

Figure 3. Effect of PE and NE infusion after SCI on microdialysis markers of cellular distress and energy metabolism (2-mm). The percentage change (%Δ) is calculated using an average of 60 minutes of baseline before SCI. (**A**) Glucose (B) glutamate (**C**) glycerol, (**D**) Lactate, (**E**) Pyruvate and (**F**) L/P ratio responses of 1-hour of vasopressor infusion (grey shading) during the compressed [0-3 hpi] and decompressed [3-6 hpi] state of the spinal cord. Decompresion time is shown by the vertical dashed line. Compared to the SCI-control group, glutamate values in the NE group were significantly ($p<0.05$) lower 0.5 hours after decompression and during the second NE infusion. For both the PE and NE group, the L/P ratio values were significantly lower 1.0 hour after decompression and onwards compared to control animals. Between the PE and NE groups, the response in L/P ratio was not significantly different at any time. Data is expressed as mean ± SEM. HPI: hours post-injury; NE; norepinephrine; PE: phenylephrine; SCI: spinal cord injury.

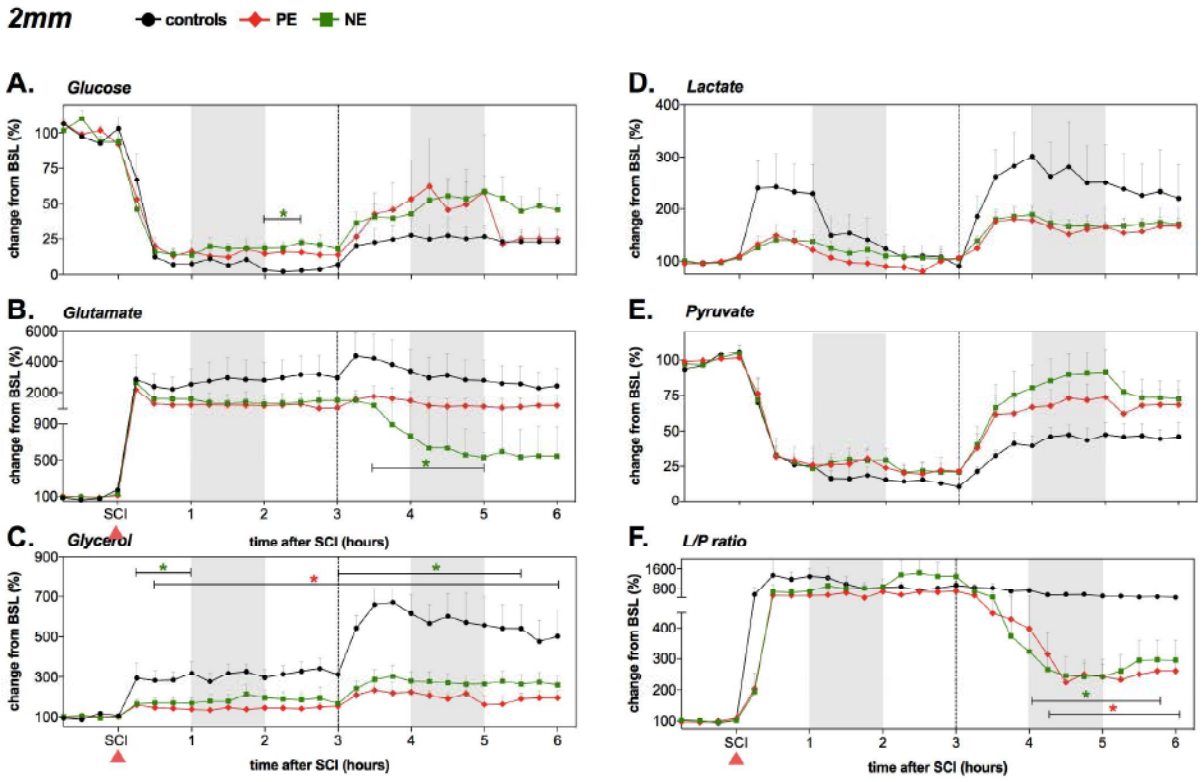

Figure 4. Effect of NE and PE infusion after SCI on blood flow, partial pressure of oxygen and pressure in the penumbra (22-mm). The percentage change (%Δ) is calculated using an average of 30 minutes of baseline before SCI. (**A**) Spinal cord blood flow (SCBF), (**B**) partial pressure of oxygen (PaO_2) and (**C**) pressure responses during 1-hour of vasopressor infusion (grey shading) during the compressed [0-3 hpi] and decompressed [3-6 hpi] state of the spinal cord. Decompresion time is shown by the vertical dashed line. Both vasopressors increased SCBF and $PaPO_2$ above pre-injury levels. Notably, SCBF continued to rise in the PE group even after infusion was ceased. Data is expressed as mean ± SEM. HPI: hours post-injury; NE; norepinephrine; PE: phenylephrine; SCI: spinal cord injury.

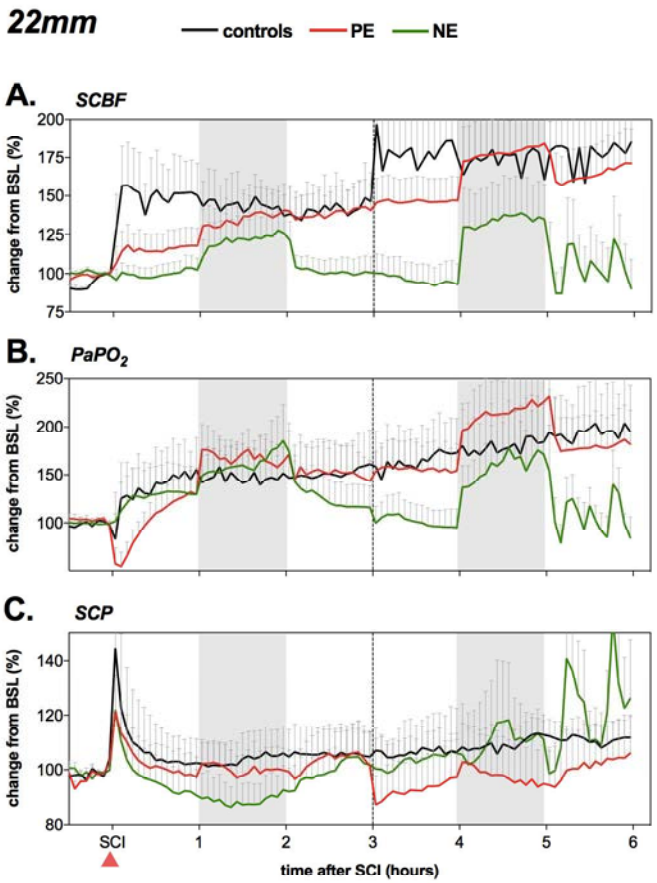

Figure 5. Effect of PE and NE infusion after SCI on microdialysis markers of cellular distress and energy metabolism (22-mm). The percentage change (%Δ) is calculated using an average of 60 minutes of baseline before SCI. (**A**) Glucose (B) glutamate (**C**) glycerol, (**D**) Lactate, (**E**) Pyruvate and (**F**) L/P ratio responses of 1-hour of vasopressor infusion (grey shading) during the compressed [0-3 hpi] and decompressed [3-6 hpi] state of the spinal cord. Decompresion time is shown by the vertical dashed line. The SCI-induced metabolic responses at the 22-mm position were far less pronounced compared to the 2-mm position. Between the experimental groups no significant differences were observed. Data is expressed as mean ± SEM. HPI: hours post-injury; NE; norepinephrine; PE: phenylephrine; SCI: spinal cord injury.